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Immunological depression in spontaneously hypertensive rats

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SUMMARY

Cell-mediated immunity was investigated in spontaneously hypertensive rats (SHR). The thymuses of young SHR rats before developing hypertension had reduced numbers of immature T lymphocytes which were detected by the rosette formation test with guinea-pig erythrocytes in the presence of foetal bovine serum, whereas the thymuses of eight other rat strains tested contained about 60% of rosetting cells. The number of rosetting cells decreased progressively with age. The blastogenic responses to PHA and Con A of the SHR rats' lymphocytes was depressed to less than one-fifth when compared to those of other rat strains including W/Mk rats, the original colony of the SHR rats. Eight-month-old SHR rats showed fewer mitogenic responses than those of 2-month-old SHR rats. Other cell-mediated immune responses, including delayed hypersensitivity, allograft rejections, and a co-operation of T and B lymphocytes to produce humoral antibody formation were depressed significantly when compared to those of other rat strains. Possible mechanisms of immunological depression in the SHR rats in relation to the development of hypertension are discussed.

INTRODUCTION

In 1963, a strain of SHR rats which develop spontaneous hypertension was isolated from Wistar rats by Okamoto & Aoki (1963). In preliminary studies to investigate T lymphocyte markers in rats we noted that SHR rats had reduced numbers of rosette-forming cells in their thymuses when compared to other rat strains (Takeichi & Boone, 1976). Additional studies were undertaken to determine more clearly whether additional impairment of immunological functions existed in this rat strain with hypertension.

MATERIALS AND METHODS

Animals. Inbred strains of Wistar-King-Aptekman/Hok (WKA/Hok), ACI/Hok and Fischer (F344/Hok) were obtained from the Institute of Experimental Animals, Hokkaido University School of Medicine, Sapporo, Japan. Inbred and closed colony spontaneously hypertensive rats (SHR) were kindly supplied by Professor H. Saito, the Department of Pharmacology, Hokkaido University School of Medicine.

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Lymphoid cell suspension. The thymus, spleen, and lymph nodes were removed from the donor animals, finely minced with scissors, and gently blended with a loose-fitting glass grinder (stock no. 1977-00005, Belco Glass Incorporated, Vineland, New Jersey) in Eagle's minimum essential medium (MEM). The crude cell suspension was passed through a cotton gauze sponge, the cells washed twice in MEM and centrifugation performed at 1,000 r.p.m. for 5 min.

Procedure for rosette formation (RF). Guinea-pig red blood cells (GpRBC) were obtained from Hartley strain guinea-pigs by cardiac puncture with heparin as an anti-coagulant. The RBC were washed twice with MEM and diluted to 0·5% in MEM (final concentration of red cells was approximately 5×10^7 cells/ml). One-tenth of a millilitre of lymphoid cell suspension was mixed with 0·1 ml of 0·5% GpRBC and 0·1 ml of 20% foetal bovine serum (FBS) (Flow Laboratory, Rockville, Maryland, USA). The mixture was incubated at 37 C for 5 min and then centrifuged at 100 *g* for 5 min at 40 C. Approximately 0·1 ml of supernatant fluid was removed, and the pellet was gently resuspended by shaking and incubated further for 60 min at 4 C. One drop of the cell suspension was placed in a haemocytometer and 200 nucleated cells were counted. Those cells which had three or more firmly attached GpRBC were considered rosette-positive.

Mitogen reactivity. Peripheral blood lymphocytes were isolated from blood obtained by cardiac puncture, using lymphocyte separation medium (LSM) (Litton Bionetics Incorporated, Rockville, Maryland, USA). Isolated lymphocytes were cultured in a flat-bottomed microtitre plate in RPMI 1640 media which had been supplemented with penicillin (50 u/ml), streptomycin (50 μg/ml), and L-glutamine (200 mM, 2 ml/100 ml of medium) plus heat inactivated 20% FBS. The mitogens, phytohaemagglutinin (PHA) (0·5 μg/well) (Difco, USA), concanavalin A (Con A) (4·5 and 9·5 μg/well) (Pharmacia Fine Chemicals AB, Uppsala, Sweden), or medium only were added to lymphocytes. The cell cultures were incubated in a 5% Co_2 incubator for 3 days. Tritiated thymidine (0·5 μCi/well) was added for the last 18 hr of culture and the cells were harvested using a microculture harvesting device. The samples were placed in 2–3 ml of aquasol (New England Nuclear, USA) and the amount of radioactivity incorporated into the acid-precipitable material was counted using a liquid scintillation counter. The arithmetic mean of the triplicate samples was determined and the results were expressed as counts per minute.

Direct plaque-forming test. The method used was similar to that described by Cunningham & Szenberg (1968). Four pieces of double-sided tape, each 6 cm wide, were laid across a clean glass slide dividing the slide into three equal portions. Pre-cleaned coverslips were placed on the tape to form shallow chambers. A mixture was made at room temperature of the lymphoid cells (1×10^7 cells/ml) under test, together with complement (1:5 final dilution of pooled guinea-pig serum), and sheep red blood cells (final concentration about 4×10^8 cells/ml). The mixture (0·1 ml/slide) was applied to the slide, and the chambers were sealed with heated paraffin. The slide was incubated at 4 C for 120 min and then at 37 C for 30 min. The number of plaques formed was counted microscopically.

Skin grafts. The technique of Kaliss & Stuter (1968) was used. Full-thickness donor skin was removed from the dorsum of Fischer/Hok rats (RTl-I). Dorsal skin which showed active hair growth was avoided. The skin was cut into circles, 10 mm in diameter, and placed in sterile MEM until transfer to the recipient site. Graft beds were prepared in a similar manner in recipients (WKA/Mk rats RTl-K, SHR rats, RTl-K). The graft bed was lightly dusted with penicillin powder and the graft positioned in place. Surgical tape (Blenderm band), 4–5 pieces 5 mm wide, and sterilized gauze were applied over the graft and polyvinyl chloride tape was wrapped around the rat. The tape was removed on day 5. The condition of the allograft was then observed on a daily basis. Rejection was signalled by reddish areas which increased to complete involvement of the graft. Survival time was recorded as the number of days to more than 50% necrosis of the graft.

Radioisotopic footpad reaction for delayed hypersensitivity. Rats were immunized intramuscularly with an emulsion of 10% suspension of erythrocytes in saline and admixed with Freund's complete adjuvant ('adjuvant' Difco). The right hind footpads of both immune and normal control rats were injected with 0·1 ml of a 10% suspension of sheep erythrocytes in saline. Immediately thereafter, ^{125}I-labelled human serum albumin (Radiochemical Centre, Amersham, England) was injected i.p. into the rats (5×10^6 c.p.m. per rat). Twenty-four hours later the rear test and contralateral control feet were cut off at the junction of the lower third of the tibia and counted in a

gamma spectrometer. Results were expressed as the foot count ratio = c.p.m./min in the test foot divided by c.p.m./min in the contralateral control foot (Takeichi & Boone, 1975).

RESULTS

Comparison of the percentage of rosette-forming cells in the thymus between SHR and other strains of rats

Table 1 shows the percentages of rosette-forming cells in the thymus and in the spleen of the eight rat strains tested. In seven rat strains, about 60% of thymus cells formed rosettes with guinea-pig erythrocytes, whereas with the SHR rat strain only 18% of thymus cells formed rosettes. W/Mk rats, the original colony of the SHR rats, had a rosette formation of 63%. None of the strains showed any rosette-forming cells in their spleens.

The influence of age upon the number of rosette-forming cells in the thymus and spleen of SHR and WKA rats

The age distribution of rosette-forming cells in the thymus and spleen was compared in SHR and WKA rats (Table 2). The incidence of cells forming rosettes in the thymus of foetal WKA rats was less than 20%, but shortly after birth the percentage of rosette-forming cells increased to 60% by 1 month of age. The number of rosette-forming cells in the thymus gradually decreased after 6 months. These results are very similar to those of F344 rats in a previous report (Takeichi & Boone, 1976). In contrast, the foetal thymus of SHR rats already had 60% rosette-forming cells and there was a rapid decrease in the cell number after birth. The foetal spleens of WKA rats contained 8% rosette-forming cells, but no rosette-forming cells were detected in the SHR foetal spleens.

Mitogenic responses of peripheral lymphocytes in SHR rats and other strains of rats

The blastogenesis of peripheral lymphocytes obtained from SHR rats and from five other rat strains to PHA and Con A is shown in Table 3. The blastogenic response of SHR rat lymphocytes was less than one-fifth that of the other rat strains including W/Mk rats, the original colony of SHR rats. Age differences in mitogenic responses in SHR and WKA rats are presented in Table 4. Lymphocytes from 2-month-old SHR rats had significantly lower blastogenic responses than lymphocytes of 2-month-old WKA rats. This difference was magnified when blastogenesis was assayed at 8 months of age. WKA rat lymphocytes increased their mitogenic responses with age, whereas the SHR rat lymphocytes were greatly suppressed.

Table 1. Distribution of rosette-forming cells in the thymus and spleen from different strains of rats

	Mean percentage of cells forming rosettes ± s.e.	
Strain of rat*	Thymus	Spleen
SHR	18 ± 2·3	0
W	63 ± 5·4	0
WKA	61 ± 5·6	0
W/F	59 ± 4·8	0
LE	63 ± 5·9	0
F344	59 ± 5·4	0
SD	62 ± 5·5	0
Donryu	60 ± 5·4	0

* One-month-old rats were used in this experiment.

Table 2. Occurrence of cells forming rosette in the thymus and spleen of WKA and SH rats

	Mean per cent of cells forming rosettes ± s.e.*			
	WKA rats		SHR rats	
Age	Thymus	Spleen	Thymus	Spleen
Foetus†	20 ± 1·7†	7 ± 1·3	60 ± 4·6	0
1 day	45 ± 2·8	0	62 ± 5·4	0
1 week	40 ± 3·4	0	37 ± 2·5	0
1 month	59 ± 4·9	0	18 ± 2·3	0
3 months	61 ± 5·8	0	28 ± 1·6	0
6 months	58 ± 4·6	0	25 ± 1·5	0
12 months	54 ± 4·1	0	26 ± 1·8	0
20 months	45 ± 3·8	0	n.t.	n.t.

* The results were obtained from three different experiments.

† Obtained from rats at 18 to 20 days of gestation.

Table 3. Blastogenic responses of peripheral lymphocytes in SHR rats and various other strains of rats

Strain of rat*	Thymidine uptake (c.p.m. ± s.e.)		
	PHA	Con A	Control
SHR	3,148 ± 331	4,265 ± 726	165 ± 34
W	23,560 ± 1,426	—	156 ± 32
WKA	20,133 ± 1,197	—	210 ± 31
ACI	18,910 ± 2,097	—	152 ± 30
Tokyo	18,465 ± 1,550	27,552 ± 3,226	84 ± 9
Buffalo	19,421 ± 3,648	50,530 ± 2,390	330 ± 80

* Four-month-old rats (three rats per group) were used in this experiment.

Table 4. Comparison of blastogenic response to PHA and Con A of lymphocytes between WKA and SH rats

Strain of rat*	Age (months)	Thymidine uptake (c.p.m. ± s.e.)		
		PHA	Con A	Control
WKA	2	7,300 ± 800	3,400 ± 400	500 ± 100
SHR	2	4,600 ± 800	700 ± 100	300 ± 100
WKA	8	23,400 ± 700	10,800 ± 900	300 ± 100
SHR	8	1,800 ± 200	1,800 ± 800	400 ± 100

* Three rats per group were used.

Direct plaque formation to sheep red blood cells in SHR rats and other rat strains

Table 5 shows the result of antibody responses to the SRBC of SHR and other rat strains using the direct plaque-forming assay. Antibody responses of the 4-month-old SHR rats were about one-tenth that of the other six rat strains.

Table 5. Comparison of antibody responses to sheep red blood cells of the SHR and other strains of rats by the direct plaque-forming assay

Strain of rat*	Mean numbers of plaques ± s.e./10^6 spleen cells
SHR	33 ± 9·5
W	385 ± 8·5
WKA	279 ± 69·3
Buffalo	273 ± 45·5
ACI	293 ± 57·6
Tokyo	329 ± 59·0
F344	327 ± 44·3

* Four-month-old rats (three rats per group) were used in this experiment.

Table 6. Delayed-type footpad responses to SRBC in WKA and SH rats

Group	Strain of rat*	Immunization with SRBC†	Foot-count ratio ± s.e.‡
1	WKA	+	1·63 ± 0·05§
2	WKA	−	1·07 ± 0·02§
3	SHR	+	1·47 ± 0·03§
4	SHR	−	1·08 ± 0·02§

* Four-month-old rats (five rats per group) were used in this experiment.

† Rats were immunized with a subcutaneous injection of 1×10^9 SRBC mixed with Freund's complete adjuvant.

‡ Group 1 *vs* Group 3, $P < 0·001$ by Student's *t*-test.

§ $P < 0·001$.

Table 7. Comparison of allograft rejection between WKA and SHR rats

Strain of recipient rat*	Donor rats	Mean survival days ± s.e.† (5 rats/group)
WKA	Fischer	6·40 ± 0·27
SHR	Fischer	7·60 ± 0·27

* WKA and SHR rats share the same major histocompatibility antigens of RT1(k).

† WKA rats *vs* SHR rats, $P < 0·02$ by Student's *t*-test.

Delayed type footpad responses to SRBC in SHR and WKA rats

Delayed hypersensitivity responses of SHR rats to SRBC were measured using the radioisotopic footpad assay. Table 6 shows that the footpad reaction of immunized SHR rats was significantly lower than that of immunized WKA rats (Group 1 *vs* Group 3).

Ability of SHR rats to reject alloskingrafts

Table 7 shows the results of allogeneic skin grafts obtained from F344 rats on SHR and WKA rats. The number of mean survival days of allogeneic skin grafts placed onto SHR rats was significantly prolonged when compared to WKA rats.

DISCUSSION

We have shown that a substantial proportion of thymus cells from many strains of rats formed rosettes with guinea-pig erythrocytes in the presence of foetal bovine serum. Elfenbein & Winkelstein (1978) and Elfenbein & Santos (1978) reported that this rosette formation was dependent on at least two factors: a high molecular weight, heat stable factor and a heat labile substance. The distribution pattern of the rosetting differs from that of θ-antigen-bearing lymphocytes in mice or that of rosette-forming T lymphocytes in humans. Recent results demonstrated that lymphocytes from spleen, lymph nodes and peripheral blood retained absorbing capacity for some components of FBS, though they did not form rosettes with guinea-pig erythrocytes (Takeichi, Suzuki & Kobayashi, 1979). This suggests that rat lymphocytes quickly lose the binding property needed to form rosettes with guinea-pig RBS when compared with that in mice or in humans.

The present experimental results showed that the absolute numbers of rosetting cells in the thymus of SHR rats was markedly reduced and that the age distribution of such cells was very different from that of WKA rats. The thymus of foetal and neonatal SHR rats contained 60% rosette-forming cells which decreased rapidly after birth. This suggests that the differentiation of the T lymphocyte series in the SHR rats may occur early in the neonatal period. The observation that the mitogenic responses of lymphocytes from SHR rats were also decreased indicates that there may be a general suppression in the cellular immune system in SHR rats. All of the cell-mediated immune responses examined, including delayed hypersensitivity to SRBC, allograft rejections and the co-operation of T and B lymphocytes to produce humoral antibody formation were significantly depressed compared to those of other rat strains.

Whether there is a correlation between the immunological depression and hypertension in SHR rats remains to be clarified. SHR rats may inherit immunological depression as well as hypertension and they may be separate and distinct diseases. Since Okamoto & Aoki (1963) isolated SHR rats from Wistar rats by consecutive brother–sister matings of the offspring with hypertension, SHR rats with immunodepression may also have been selected inadvertently. One- to two-month-old SHR rats did not yet have spontaneous hypertension, but immunological depression was observed. Some of the aged SHR rats which did not develop hypertension had reduced immune responses. One could speculate that the immunological depression in SHR rats may be in some way linked to the development of spontaneous hypertension. In this regard, it is of interest to see that the grade of immunological depression in SHR rats was increased by ageing and the aged SHR rats which had severe hypertension also had strongly reduced immunity. Aoki (1964) reported that histopathological studies of the SHR rats showed hypertrophy of the pituitary, adrenal and thyroid glands (Aoki *et al.*, 1963; Aoki, 1964). He also demonstrated that the removal of these endocrine organs from SHR rats inhibited the development of hypertension and that the administration of cortisone or thyroid powder accelerated the development of hypertension in SHR rats. It is easy to predict that some of the changes seen in the endocrine organs may play a significant role in the development of the immunological depression observed in our study.

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